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14. ABSTRACT

The contractor conducted research to: establish the magnitude and duration of sleep inertia after waking from short episodes of sleep; determine how the level and time course of sleep inertia varies according to circadian phase; and determine how the level and time course of sleep inertia varies according to the duration of prior waking. The specific objectives were:
1. To determine the magnitude of any decrements in performance and subjective and/or neurophysiological alertness upon waking from naps ending at 0200 hrs (after about 20 hrs awake), compared to the 'no nap' condition,
2. To determine the time course for any decrements to return to baseline levels (compared to the no-nap condition).
3. To determine whether the nap (duration, proportion of SWS or sleep stage on awakening) influences the magnitude or time course of sleep inertia effects under these conditions.

15. SUBJECT TERMS

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Time Course and Magnitude of Sleep Inertia

Grant Number: FA4869-06-1-0041
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Final Progress Report
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Introduction

This final report provides an update on progress on the AOARD funded study (FA4869-06-1-0041 AOARD 06400), presently being conducted at the Sleep/Wake Research Centre, Massey University. The aim of the study is to accurately document the magnitude and time course of sleep inertia after naps of 20, 40 or 60-minutes duration, taken after 20 hours of wakefulness and at 0200 in the circadian cycle.

Sleep Inertia

The transient confusion and reduced cognitive and behavioral performance seen on sudden awakening is known most commonly as sleep inertia (1, 2). Sleep inertia is one of the most serious contraindications to the use of napping in operational settings, particularly if an individual may be required to perform complex tasks immediately after sudden awakening. It has also been noted as a possible cause of air accidents (3). The physiological underpinnings of sleep inertia are still unclear, but it is hypothesized to be due to a gradual neurophysiological transition between sleep and wakefulness, which takes time to be completed (1).

Attempts have been made to incorporate sleep inertia into different theoretical models of alertness and performance (4-7). The two other major components in these models are circadian variation (process C) and the homeostatic sleep drive (process S), which depends on prior sleep/wake history. Data from a number of neurobehavioral measures indicate the amplitude of the circadian variation is dependent on the level of the homeostatic sleep drive (8-10) indicating that S and C interact in a non-linear manner. It has also been suggested (11) that sleep inertia (process W) may interact in a non-linear manner with S and C, although in current models it is treated as acting independently. For example, the duration and magnitude of sleep inertia may increase when people are sleep deprived (implying an interaction of W and S). It is also possible that sleep inertia is worse at some circadian phases than at others (implying an interaction of W and C).

Better data are needed to clarify these possible interactions, and to provide improved guidelines for the effective use of napping as a fatigue countermeasure in operational settings.

Duration of Sleep Inertia

To date, findings of previous studies aimed at quantifying the magnitude and time course of sleep inertia have been inconsistent. This is in part due to disparities in methodology, such as differences in duration of prior wakefulness, differences in duration and timing of naps, and differences in the performance tests used and the frequency of testing.

Without prior sleep loss, the duration of sleep inertia has been reported by some authors to be up to 20 minutes (12-14), while other studies report no measurable effects after a minute (15). Jewett et al. (11) demonstrated that, following wake at the habitual time from a full 8-hour sleep episode, the effect of sleep inertia on cognitive performance (2-minute addition task) levelled off in an asymptotic manner only after 2 hours. Achermann et al. (16) reported that the effect of sleep inertia on reaction time, after awakening from night time sleep or an evening nap, subsided over 30 minutes but persisted for up to 1 hour.

Magnitude of Sleep Inertia: Effect of Sleep Stage and Sleep Duration

Early laboratory studies indicated that sleep inertia was more severe after waking from slow wave sleep (12), and after spending longer in slow wave sleep (SWS) (17), possibly due to the greater neurophysiological transition required from sleep to wakefulness. However this finding is not universal (18, 19).

Sleep inertia is also reported to be most severe with, and last for longer, after greater prior sleep loss (20), which would be expected to increase the homeostatic drive for SWS.

Magnitude of Sleep Inertia: Effect of Circadian Phase

The magnitude of sleep inertia is also thought to be affected by circadian phase and is reported to be greatest near the nadir of core body temperature (2, 21), when the circadian drive for sleep is greatest.

Objectives of Present Study

The present study is designed to maximise the effects of sleep inertia, assessed through measures of cognitive performance, neurophysiological alertness, and subjective reports. The likelihood of sleep inertia is maximised by creating a high homeostatic drive for sleep by extending wakefulness to approximately 20 hours and

testing sleep inertia close to the circadian nadir, by waking the participant from a nap at 2am.

The objectives of the present study are to:

1. Determine the magnitude of any decrements in performance and subjective and neurophysiological alertness upon waking from naps ending at 02:00 hrs (after 20 hrs awake), compared to when no nap is taken.

2. Determine the time course for any decrements in waking functioning to return to baseline levels (compared to when no nap is taken).

3. Determine whether the nap (duration, proportion of SWS or sleep stage on awakening) influences the magnitude or time course of sleep inertia.

Method

Recruitment and Screening

For the first 3 runs of the study nine healthy adult males were recruited from the Wellington (NZ) region. To reduce the likelihood of confounding caused by changes in sleep across the menstrual cycle, all participants were male. Participation was informed and voluntary and conformed to all institutional and government guidelines for the protection of human subjects. Written consent was obtained from each participant. The following selection criteria were used.

- Age 20-35 years.

- Non- or mild/moderate caffeine consumers (0-3 cups per day). Participants were asked to minimize caffeine consumption for 7 days prior to the laboratory protocol, and to avoid caffeinated drinks from midday the day before the laboratory protocol.

- Non-smokers.

- Non- or social drinkers only (0-10 standard drinks per week).

- No shift workers.

- No previous diagnosis for psychiatric and/or neurological problems.

- Not taking any medication/drugs acting on central nervous system. A urine drug screen test was performed when participants arrived at the research facility each weekend for the laboratory protocol.

Participants were recruited through advertisements in local print media, under the heading: "The effects of napping on performance and alertness during a period of sleep deprivation". There was no mention of sleep inertia to the participants at any stage of the study, thus reducing the likelihood that they could react to the demand characteristics of the study.

Participant screening involved completing a questionnaire and meeting certain criteria on a number of standardised scales; completing and passing a medical examination; and completing and passing a urine drug test prior to each weekend protocol. Further detail on the screening process can be found in Appendix A.

Prior to the Laboratory Protocol

Each laboratory protocol was preceded by 7 nights of stable sleep, monitored with an Actiwatch™ (Minimitter™) and sleep diary. The Actiwatch™ is a small watch-sized device, which measures the occurrence and degree of wrist motion with an accelerometer. This device has been proven highly sensitive to sleep, with correlations of approximately 0.96 to polysomnographically recorded sleep in both normal and sleep disordered populations (22, 23). In addition to wearing an Actiwatch™, participants completed a sleep diary, to provide reference information for the actigraphy output. The sleep diary was developed from log books used in previous studies (24-28).

To reduce disrupted sleep caused by wearing the polysomnographic recording equipment for the fist time (first night effect), participants wore the recording equipment for one night's sleep at home (adaptation night), prior to their first laboratory protocol.

During the Laboratory Protocol

Participants were involved in the protocol on 4 separate occasions; one for each nap condition plus the control condition of no nap. Participants were reimbursed for their time and effort for each of the 4 study protocols, plus a bonus at the end of the study. Taxi chits were provided for transport to and from the research facility.

Protocols were conducted during the weekend and commenced on a Friday evening and finished on a Sunday morning (see Figure 3). During this time participants were asked not to leave the premises and no vigorous exercise was allowed. Participants slept and undertook all testing in their own cubicle in the Sleep Laboratory. This facility allows up to three participants to be studied in an environment where temperature and lighting can be controlled. Research Assistants monitored the participants throughout the study protocol to ensure that they remained awake during times that were not scheduled sleep periods. Sleep periods were monitored to ensure recording quality was maintained and in case a participant required assistance.

Iso-caloric meals were scheduled at regular times (see Figure 3), and snacks and non-caffeinated drinks were provided regularly throughout the protocol. Meals were balanced in terms of carbohydrate and protein content and snacks with high sugar content were not provided.

Each participant had access to a colour television and game console/DVD player, with a selection of games and movies as aids to remain awake and entertained in between test sessions.

The study design involved manipulating the length of the nap opportunity (20, 40, or 60 minute naps) to influence the proportion of slow-wave sleep obtained and the sleep stage from which people woke. Even under conditions of extended prior wakefulness, the likelihood of entering and spending time in SWS during a 20-minute nap opportunity is expected to be small. A 40-minute nap increases this likelihood, while during a 60-minute nap opportunity an individual is expected to spend some time in SWS. All nap opportunities ended at 0200 hours and were immediately followed by a 6-minute test battery, repeated at a high rate for the first 3 hours following waking (see Figure 3).

Protocol

Participants arrived at the Sleep Laboratory at 1800 hours on Friday and were provided with time to settle in prior to providing a urine sample for drug screening. On the first of the 4 weekend protocols, participants completed the remaining performance test practice sessions and were also familiarised with the Psychomotor Vigilance Task (PVT).

Participants had electrodes placed on their head and face to record sleep and waking alertness throughout the remainder of the protocol (using the A10 ambulatory recorder, Embla™). Gold Grass electrodes were placed according to the 10-20 system at EEG sites: C4, C3, Fz, Pz and Oz (referenced to A1 or A2). Left and right EOG was recorded from the left and right outer canthus, again referenced to A1 or A2. Bipolar EMG was recorded from 2 electrodes positioned on the mentalis/submentalis muscles.

Prior to the night sleep opportunity commencing, instructions for the first test battery the following morning were read. Lights were switched off at 2200 hours. This initial night of sleep allowed the amount of sleep obtained, and the duration of wakefulness preceding performance testing, to be as uniform as possible across protocols.

At 0600 hours the following morning an alarm clock inside the Sleep Laboratory sounded. A Research Assistant switched on the lights, entered the room, and switched off the alarm. Participants were required to immediately leave their beds, sit on a chair

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located in their cubicle and commence the first test battery. If the participants did not rouse after the alarm or failed to start the test battery they were gently reminded by a Research Assistant of the correct procedure. Throughout all test batteries Research Assistants monitored the behaviour of participants.

On completion of the first test battery participants were provided with breakfast. Following the second test battery (0800 hours, see Figure 3) participants were given the opportunity to shower and have the electrodes reapplied. The timing of the further test batteries prior to the nap can be seen in Figure 3.

Prior to a nap opportunity commencing, participants were reminded of the order of events following the nap and the quality of the electrophysiological connections were checked. Depending on the duration of the nap, the lights were switched off at either 0140 hours (20-minute nap), 0120 hours (40-minute nap), or 0100 hours (60-minute nap).

To accurately track the time course of sleep inertia, intensive testing occurred immediately following waking at 0200 hours (waking from the nap followed the same procedure as at 0600 hours the previous morning). For the first hour, 2 test batteries were completed back to back every 15 minutes. From 0300 hours to 0500 hours 2 test batteries were completed back to back every 30 minutes, then single test batteries were completed hourly until the protocol ended at 0800 hours. See Figure 3 for further detail.

Following the final test battery participants were provided with breakfast, given a small financial compensation for their time, a blank sleep diary for the week preceding the next protocol, and provided with a taxi to get home.

Test Battery

A short (6-minute) test battery was utilised so that testing could be quickly and easily repeated. During the intensive testing period immediately post-nap, rest periods were required to prevent time-on-task effects confounding the findings (12-14). For the first hour of testing, a 3-minute rest followed each two 6-minute test battery (see Figure 2). Any subsequent decrements in performance and/or alertness were therefore expected to be due to sleep inertia. Each test battery comprised 3 measures: a rating of subjective alertness; a working memory task; and the measurement of neurophysiological alertness.

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To provide information that can be compared to findings from previous studies on sleep inertia, e.g..(11), each test battery commenced with a rating of subjective sleepiness. The Karolinska Sleepiness Scale (KSS) is a widely-used scale, that has been validated with respect to decrements in performance and objective measures of sleepiness (29, 30). The KSS is a 9 point scale from 1 (extremely alert) to 9 (extremely sleepy, fighting sleep), that takes approximately 30 seconds to complete.

Working Memory (N-back task)

To accurately document the magnitude and time course of sleep inertia on performance, the N-back task, a 4-minute working memory task, was employed (31-36). This task was chosen for the following attributes:

- previous findings showed that the effects of sleep inertia are more obvious when high attentional load is required such as in working memory tasks (2, 37);

- in contrast to self-paced tasks, work-paced tasks are much less likely to be confounded by a change in cognitive strategy, often trading accuracy for speed, and are thus likely to be highly sensitive to relatively small changes in sleep inertia;

- the short, 4-minute duration of the task allows for frequent testing, thus improving the measurement of the time course of sleep inertia;

- in a similar fashion to sleep deprivation, sleep inertia causes a general decline in the ability to allocate attention to a task (1) and because sleep deprivation leads to increased visual and auditory distraction due to a lack of focused attention (38), it is expected that sleep inertia will also lead to similar difficulties in avoiding distractions;

- the test has been well-validated.

The task requires participants to compare the spatial location of a "current" stimulus with the nth-item back (e.g.: 1-back, 2-back) in a sequentially presented list of items. The participant must judge whether these locations match or not, regardless of their identities. The task challenges individuals to maintain the n-back item in focal attention while concurrently processing new items. Substantial demands are placed on

executive processes because the response set must be continually updated as a new stimulus is presented, by focusing on the spatial location of the stimulus while disregarding its identity.

In the present study, the 2-back task was utilised, rather than the simpler 1-back task, to maximize the sensitivity to the effects of sleep inertia. In the 2-back task, one of 12 possible capital-letter stimuli appears in one of 12 possible locations on a computer monitor. Participants compare the location of the current stimulus, regardless of its identity as a letter, with the location of the stimulus presented 2 trials previously (see Figure 1). That is, participants are required to maintain two positions and their sequential order in working memory for the duration of two trials, and to update that information on each trial. Since each trial lasts 4.5 seconds, accurate performance in these conditions require that stimuli are retained for ~ 13.5 seconds. Participants are instructed to respond as quickly and as accurately as possible, and to indicate match detection with an index finger key press, and a non-match with the middle finger (both of the dominant hand).
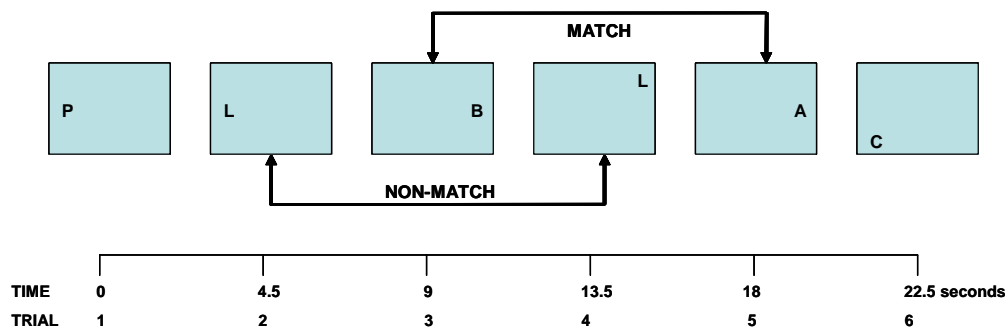
Figure 1. Representation of the 2-Back task.

The single, capital-letter stimuli, drawn randomly from a set of 12, are presented for 200 milliseconds, once every 4.5 seconds. At 1.3 seconds prior to stimulus onset, a warning cue appears on the screen for 200 milliseconds. The letter stimulus occurs 1.3 seconds after the cue in 1 of 12 possible locations on the monitor. The identity of the letter and its spatial position vary randomly from trial to trial. Matches occur on 50% of the trials. A test block, consisting of 50 trials, preceded by 3 warm-up trials (to be discarded from analysis), has a duration of 4 minutes. The test is completed by the participant on a laptop and is run by LabVIEW™ software (National Instruments™). The outcome measures used in the present report are: total number of correct matches

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and correct non-matches; mean reaction time for correct matches and correct non-matches; and number of omissions (failure to respond prior to the next stimulus being presented).

To remove practice effects, participants completed 40 practice tests (2000 trials) prior to their involvement in the protocols. Asymptotic performance is reported to be achieved after 200 trials (where 1 trial = 1 stimulus) (35) but further information from M. Smith (personal communication, September 28, 2005) suggests that participants in their study had in fact around 2000 trials of practice on the 2-back task. Practice sessions were completed in association with the initial screening meeting (750 trials), either the medical or adaptation night (500 trials), and the Friday night of the first weekend protocol (750 trials).

90-second EEG recording

The test battery also included a 90 second controlled EEG recording with eyes open. In addition to the sites used for recording sleep (C3 and C4), electrodes were placed at Fz, Pz and Oz to detect changes in neurophysiological alertness.

EEG recordings have been used extensively to obtain objective neurophysiologic measures of changes in alertness, with the majority of studies focusing on the transition from wakefulness to sleep (29, 39, 40). Using EEG indices, Pigeau, Heslegrave et al. (41) found that sleep inertia resembles an early phase of sleep. Ogilvie and Simons (10) measured changes in EEG spectral power during the transition from sleep to wakefulness and found that delta, theta and sigma (the three 'sleep' frequencies) decreased as the participant awakened and maintained wakefulness. Beta activity tended to increase gradually and linearly across the transition time, whereas alpha first increased and then decreased to levels similar or below those seen in sleep. The EEG changes occurred more slowly during sleep offset than during sleep onset.

A further argument for including EEG spectral power analysis comes from the study of Smith et al. (31). The authors demonstrated that combining EEG spectral characteristics with the performance measures resulted in a monotonic increase in discriminability from baseline. In other words, using analysis of performance measures alone, 69 % data samples could be accurately discriminated from the baseline measure. In the combined analysis, 94% of the data samples could be accurately discriminated from baseline.

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EEG data collected in the present study will be visually scored for artefact and the power spectrum of clean epochs calculated using a purpose-built Matlab™ program.
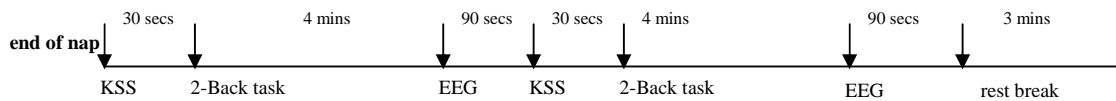
end of nap	30 secs		4 mins		90 secs	30 secs	4 mins		90 secs		3 mins
	KSS		2-Back task		EEG	KSS	2-Back task		EEG		rest break

Figure 2. Schematic representation of two test batteries followed by a 3-minute rest break.

Psychomotor Vigilance Task (PVT)

Changes in alertness and performance across the protocol were also assessed using the Psychomotor Vigilance Task (PVT) (42). The PVT is widely used in studies of fatigue, sleep deprivation, and performance and has been shown to be sensitive to changes in alertness associated with circadian phase (8, 43).

The test requires individuals to respond as rapidly as possible to the presentation of numerals on a 4 digit LED numeric display. It is a "simple" reaction time test, in that it does not involve a choice between responses but does provide feedback on performance via the numerals on the LED display. The test length was set at 10 minutes, inter-stimulus periods were able to range between a minimum of 2000 milliseconds and a maximum of 10000 milliseconds, and a lapse was categorised as any response longer than 500 ms (which is twice that of the mean response time). These values are the standard test parameters used in the validated version of the PVT (44).

The test was initialised so that only the button associated with the individuals dominant hand was activated for responding. Each subject completed a 1 minute trial of the PVT prior to the study. The PVT shows no practice effects (45), therefore a brief trial of the test was sufficient to familiarise subjects with the structure of the test before data collection began. Due to the length of the test, the PVT was only completed during test batteries prior to the nap and those occurring from 0300 hours onwards.

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Figure 3. Protocol timetable

TIME	TASK	1	2	3	4
1600	Staff Arrive				
	SETUP				
Karl	-Tidy Communal areas/bathrooms etc.				
	-Ensure that all dishes/cutlery are clean				
	-Lab bedding and furniture in place				
Karl	-Set and record lab illumination and temperature				
	-Set up staff bed				
	-Set up and test DVD and television equipment/batteries				
	-Water pitchers, glasses, menus in place				
Karl	-Set up and label equipment for drug screening				
Karl	-Set up PVT-label/hand to use				
Karl	-Ensure cubicles and all other equipment are labelled				
1800	Participants arrive				
Karl	-Urine tests				
	-Give the participants time to settle in				
Karl	-DVD requests/rentals				
1840	N Back Practice 3 (5 tests)				
1900	Meal 1				
	Menu: Dinner				
1930	N Back Practice 4 (5 tests)				
1950	PVT Practice				
2000	Set up sleep recordings				
2100	N Back Pratice 5 (5 tests)				
2200	Start baseline sleep				
0600	Test Battery 1				
	Karolinska 1				
	N Back Task 1				
	EEG 1				
	PVT 1				
0700	Meal 2				
	Menu: Breakfast				
0800	Test Battery 2				
	Karolinska 2				
	N Back Task 2				
	EEG 2				
	PVT 2				
0815	Participant 1				
	Shower				
	Electrodes Reapplied				

TIME	TASK	1	2	3	4
0830	Participant 2				
	Shower				
	Electrodes Reapplied				
1000	Meal 3				
	Menu: Morning Tea				
1030	Participant 3				
	Shower				
	Electrodes Reapplied				
1200	Test Battery 3				
	Karolinska 3				
	N Back Task 3				
	EEG 3				
	PVT 3				
1230	Meal 4				
	Menu: Lunch				
1330	Download actiwatches				
1500	Meal 5				
	Menu: Afternoon Tea				
1600	Test Battery 4				
	Karolinska 4				
	N Back Task 4				
	EEG 4				
	PVT 4				
1830	Meal 6				
	Menu: Dinner				
2000	Test Battery 5				
	Karolinska 5				
	N Back Task 5				
	EEG 5				
	PVT 5				
2200	Test Battery 6				
	Karolinska 6				
	N Back Task 6				
	EEG 6				
	PVT 6				
2300	Meal 7				
	Menu: Supper				
2315	Test Impedences				
2400	Test Battery 7				

TIME	TASK	1	2	3	4
2400	Karolinska 7				
	N Back Task 7				
	EEG 7				
	PVT 7				
0045	Participants in bed for condition 4				
0100	Condition 4				
0105	Participants in bed for condition 3				
0120	Condition 3				
0125	Participants in bed for condition 2				
0140	Condition 2				
0200	Condition 1				
0200	Wake participants				
0200	Test Battery 8				
	Karolinska 8				
	N Back Task 8				
	EEG 8				
	Test Battery 9				
	Karolinska 9				
	N Back Task 9				
	EEG 9				
0215	Test Battery 10				
	Karolinska 10				
	N Back Task 10				
	EEG 10				
	Test Battery 11				
	Karolinska 11				
	N Back Task 11				
	EEG 11				
0230	Test Battery 12				
	Karolinska 12				
	N Back Task 12				
	EEG 12				
	Test Battery 13				
	Karolinska 13				
	N Back Task 13				
	EEG 13				
0245	Test Battery 14				
	Karolinska 14				
	N Back Task 14				

TIME	TASK	1	2	3	4
0245	EEG 14				
0245	Test Battery 15				
	Karolinska 15				
	N Back Task 15				
	EEG 15				
0300	Test Battery 16				
	Karolinska 16				
	N Back Task 16				
	EEG 16				
	Test Battery 17				
	Karolinska 17				
	N Back Task 17				
	EEG 17				
	PVT 8				
	Karolinska 18				
0330	Test Battery 18				
	N Back Task 18				
	EEG 18				
	Test Battery 19				
	Karolinska 19				
	N Back Task 19				
	EEG 19				
	PVT 9				
0400	Test Battery 20				
	Karolinska 20				
	N Back Task 20				
	EEG 20				
	Test Battery 21				
	Karolinska 21				
	N Back Task 21				
	EEG 21				
	PVT 10				
0430	Test Battery 22				
	Karolinska 22				
	N Back Task 22				
	EEG 22				
	Test Battery 23				
	Karolinska 23				
	N Back Task 23				

TIME	TASK	1	2	3	4
0430	EEG 23				
	PVT 11				
0500	Test Battery 24				
	Karolinska 24				
	N Back Task 24				
	EEG 24				
	PVT 12				
0600	Test Battery 25				
	Karolinska 25				
	N Back Task 25				
	EEG 25				
	PVT 13				
0700	Test Battery 26				
	Karolinska 26				
	N Back Task 26				
	EEG 26				
	PVT 14				
0800	Test Battery 27				
	Karolinska 27				
	N Back Task 27				
	EEG 27				
	PVT 15				
0830	Meal 8				
	Menu: Breakfast				
0835	Taxis 04 384 4444				
	Book for 0900				
0900	Participants				
	Cheques/chits				
0905	Participants go home				
Sunday	**CLEAN UP**				
	-strip beds, researcher bed, linen to Launderette				
	-food is tidied, dishwasher on				
	-put DVDs in cases, return rentals				
	-PVTS off, lights, TVs, Xboxes off				
Monday	**CLEAN UP**				
	-empty Dishwasher				
	-pick up laundry				
	-air TIU facility-open doors				

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Results

The following results are provisional and are derived from 7 participants who completed the first 3 data collections runs. Data presented here include nap sleep recordings, ratings of subjective sleepiness, and short-term memory performance. Analysis of the 90-second EEG recordings, psychomotor performance and sleep during the first night of the protocol will be included in the final report. The statistical analyses detailed here are intended to provide an example of the type of analyses that will be conducted on the final dataset. Because of the small dataset used for the final report these multivariate analyses may not be reliable.

Participants

Across the first 3 runs of the study 22 individuals enquired about participating in the study and 21 completed the initial screening questionnaire. Eleven individuals met the screening questionnaire criteria and passed the medical examination. Of these, 9 were involved in the first 3 runs of the study. However, only 7 participants (mean age=27, range=20-35) completed all 4 study weekends. Two participants withdrew from the second run for reasons beyond the control of the researchers (changed work commitments and personal circumstances).

Table 1 details the number of participants completing each study condition on the first, second, third or fourth study weekend. Due to the small number of participants in the first 3 runs, the number completing each condition on each weekend are not balanced and therefore the results presented here may be influenced by the order in which conditions were completed. In the final dataset the order in which study conditions were completed will be balanced.

Table 1. Number of participants completing each study condition by weekend

Condition	1st weekend	2nd weekend	3rd weekend	4th weekend
No nap	3	-	3	1
20-minute nap	1	3	3	-
40-minute nap	3	1	-	3
60-minute nap	-	3	1	3

The order in which each run completed the 4 study conditions was as follows:

- Run 1: no nap, 60-minute nap, 20-minute nap, 40-minute nap.

- Run 2: 20-minute nap, 40-minute nap, 60-minute nap, no nap.

- Run 3: 40-minute nap, 20-minute nap, no nap, 60-minute nap.

Nap Sleep

Final scoring of the nap sleep is presently underway, with the following noted regarding sleep duration and structure. All participants slept during all of the nap opportunities provided and latency to the first 60-seconds of sleep is, for the large majority, very short. Of the nap recordings that have been scored all participants, except one, had sleep latencies of less than 5 minutes. The remaining participant took 6.5 minutes to fall asleep. Participants remained asleep for the duration of the nap, with wake time during the sleep period being less than 1-minute, with one exception (wake time of 3.5 minutes).

All of the 20-minute naps have been scored for the first 7 participants. Total sleep time (TST) during this nap ranged between 14.5 and 19.5 minutes and sleep was largely non-rapid eye movement sleep (NREM) stages 1 and 2 (S1 and S2). A small amount of slow wave sleep (SWS) was noted in 4 of the 7 participants (2 minutes or less). No rapid eye movement sleep (REM) was seen in any of the 20-minute nap opportunities.

During the 40-minute nap, TST was 35 minutes or greater (for the 4 recordings scored to date). Compared to the 20-minute nap, greater amounts of SWS were seen (1-18 minutes) and for one participant a small amount of REM sleep occurred (2 minutes).

Total sleep time during the 60-minute nap ranged between 53 and 59.5 minutes (for the 3 recordings scored to date). In contrast to the 40-minute nap, more SWS and REM sleep was scored.

Subjective Sleepiness (Karolinska Sleepiness Scale)

Missing Data

One data point out of 756 was missing. This was a single rating from 1 participant during test session 17 on the no nap weekend.

Raw Data

Mean ratings of subjective sleepiness, as measured by the KSS, across each study condition are plotted in Figure 4[1]. Each graph displays circadian variability in subjective sleepiness, with a decline in sleepiness across the daytime (test battery 1-4, 0600-1600), generally followed by increasing sleepiness across the late afternoon and night. Ratings of subjective sleepiness appear to be lower and remain at a stable level following a 60-minute nap (test battery 8 onwards), compared to all other conditions. Subjective sleepiness is high following a 40-minute nap, but like the 60-minute nap sleepiness remains stable across the remainder of protocol, while the 20 minute nap appears to have less effect on subjective sleepiness (i.e. sleepiness increases in a similar manner to that seen in the no nap condition).

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[1] Data at each test time were not always normally distributed

No Nap Condition

40-minute Nap Condition

20-minute Nap Condition

60-minute Nap Condition
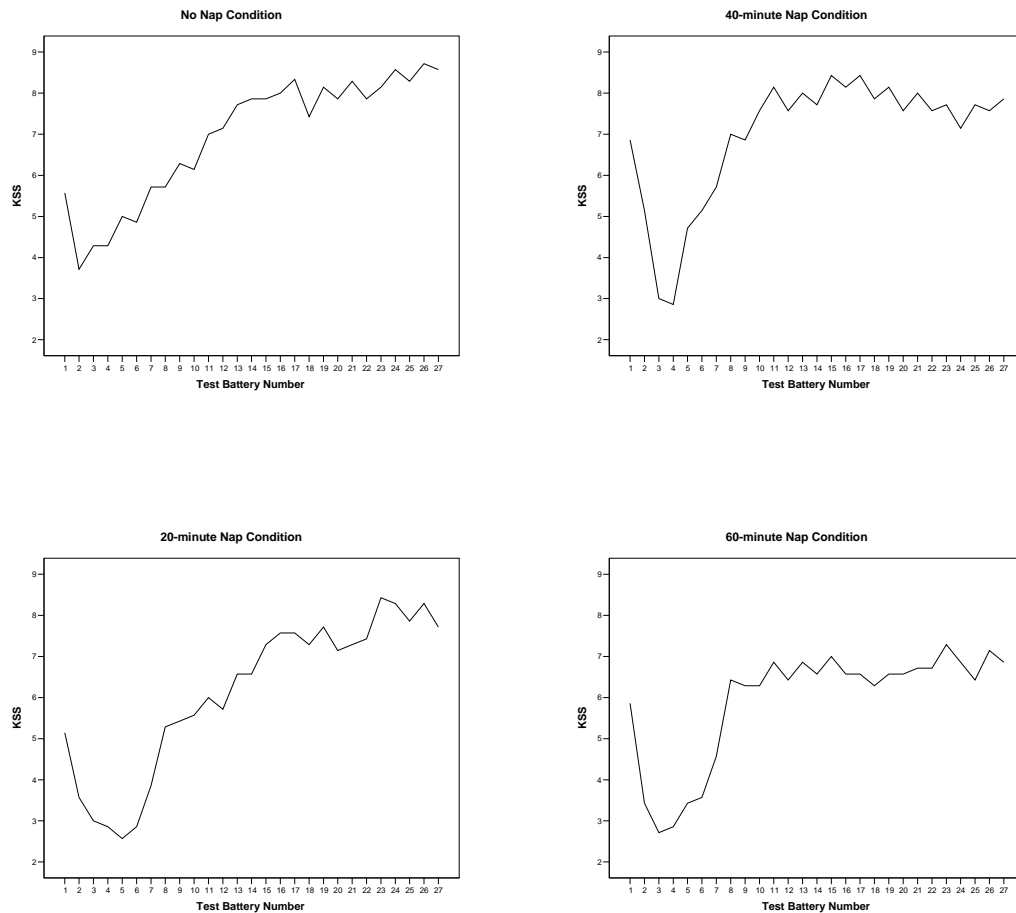
Figure 4. Mean ratings of subjective sleepiness across the study protocol

Differences from No Nap Condition

To remove the circadian variation in subjective sleepiness, at each test time point the difference between sleepiness when a nap was provided and when no nap was provided was calculated. These differences are plotted in Figure 5.

Results indicate that immediately following a 20-minute nap, participants feel less sleepy compared to when no nap was provided, but that this effect decreases somewhat over time. Following both a 40-minute and 60-minute nap, participants initially rate themselves as sleepier than when no nap was provided. This effect appears to be greater and last for longer following the 40-minute nap. At around 3:30am following a 40-minute nap, ratings of sleepiness improve relative to the no nap condition and participants remain less sleepy than when no nap was provided.

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The effect is similar following a 60-minute nap, but participants feel less sleepy compared to the no nap earlier (around 2:30am) and this continues until the end of the test session (8am).
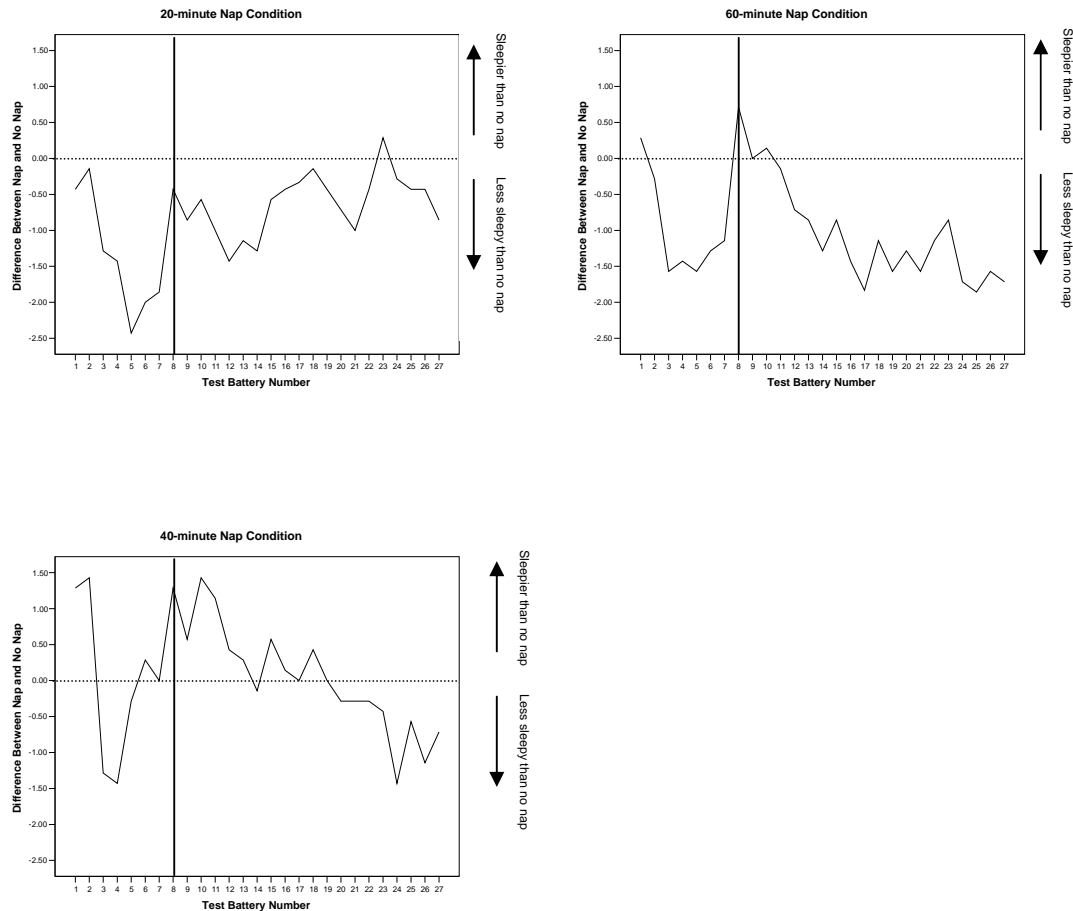
Figure 5. **Differences in subjective sleepiness between nap and no nap conditions**

Differences at Each Test Time

Mixed model ANCOVAs were conducted to determine if sleepiness ratings varied according to nap duration at each test time. As plotted in Figure 5 above, the dependent variable was the difference in sleepiness ratings between the nap and no nap condition at each test session, and independent variables included nap duration (20, 40, 60 minute nap) and the order in which study conditions were completed (1st, 2nd, 3rd, or 4th).

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The duration of the nap significantly affected sleepiness ratings only at 2200 (test 6), prior to a nap being taken. At this time and all other test times prior to the nap, model estimates indicated that sleepiness tended to be lower in the 20-minute and 60-minute nap condition compared to the no nap condition, and higher in the 40-minute nap condition compared to the no nap condition. Sleepiness was consistently lowest in the 20-minute nap condition compared to the 40-minute and 60-minute nap, and consistently highest in 40-minute nap condition.

Following the nap, model estimates indicated that sleepiness in the 20-minute nap condition was consistently lower than in the no nap condition, other than at approximately 0437 (test 23). In the 40-minute nap condition sleepiness was greater than when no nap was taken, except at 0245 (test 14) and from 0400 onwards (test 20-27). In the 60-minute nap condition sleepiness was only greater than when no nap was taken at 0200 (test 8). At all other test times sleepiness was lower.

Between 0200 and 0300 sleepiness was lower following a 20-minute nap compared to a 60-minute nap, and highest following the 40-minute nap. From 0300 onwards sleepiness was lower following the 60-minute nap compared to the 20-minute nap. Only at 0437 and 0500 (test 23 and 24) was sleepiness lower after a 40-minute nap compared to the 20-minute nap.

It must be reiterated that none of these results (other than those for test 6) were statistically significant.

Working Memory Performance

Missing Data

One participant consistently completed the task by using a single key to respond to all events. As a consequence, his data has been excluded from all analyses and results are reported for the remaining 6 participants. During the 20-minute nap condition, technical issues resulted in missing data for 3 tests (test time 1, 2 and 3) for 1 participant.

Practice Effects

Data for each participant across all 148 n-back tests completed during both the 40 practice sessions and on each consecutive weekend were plotted (see Figure 6) to determine when performance plateaued. For 4 of the 6 participants, the plots indicate

that performance plateaus at or before the 40 practice tests are completed. One individual's performance improves considerably after the practice sessions are complete, while another's performance declines. For both these individuals their performance then appears to stabilise at the new level. For some individuals circadian variability in performance is apparent and for 4 of the 6 participants, performance appears to decline from one weekend to the next (regardless of the study condition). This decline across consecutive weekends suggests a need to control for the order in which study conditions were completed.
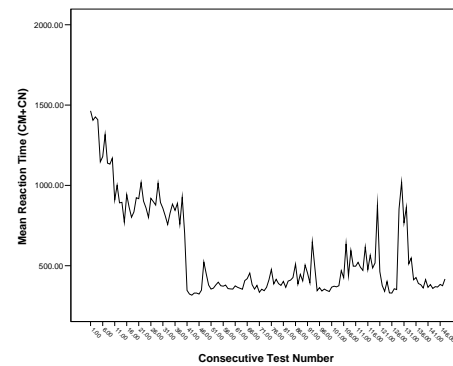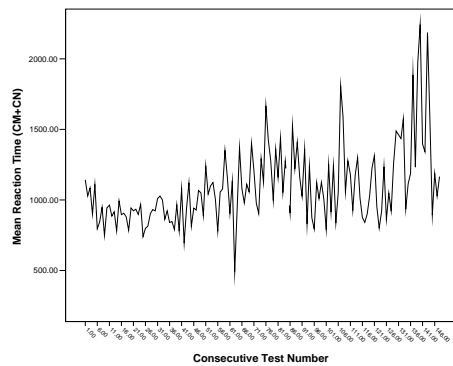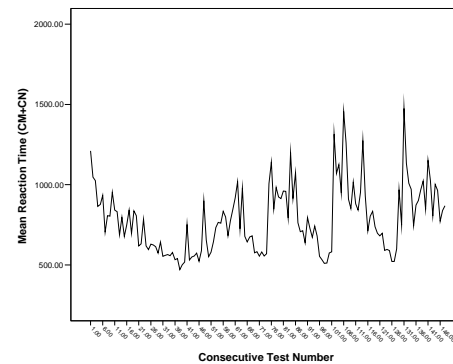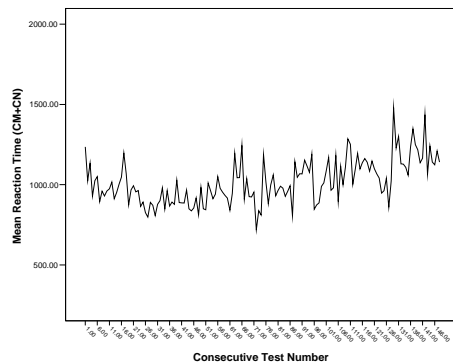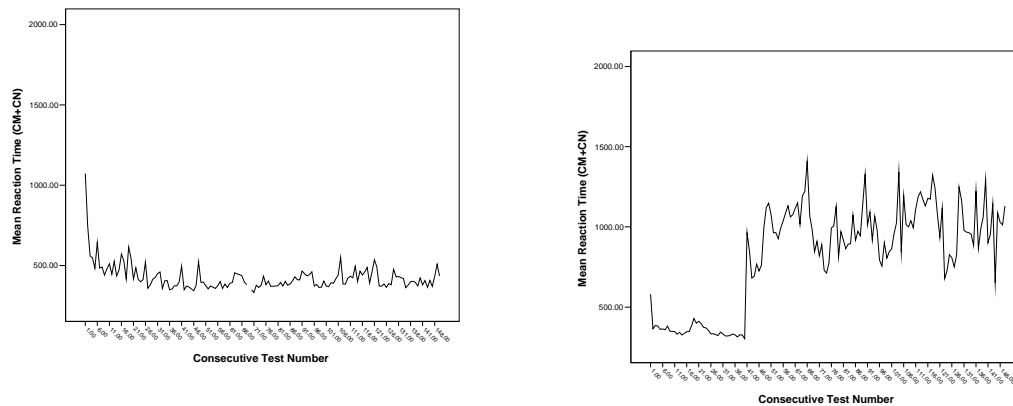
Figure 6. Mean reaction time (CM+CN) for all consecutive N-back tests completed by each participant.

Raw Data

Means of the total number of correct matches and non-matches, mean reaction time (correct matches plus correct non-matches), and mean number of omissions for each study condition are plotted in Figure 7[2]. Circadian variability in performance is apparent in the no nap condition for each performance variable. Performance following the 20-minute nap is relatively stable in comparison to all other conditions, while immediately post the 40-minute and 60-minute nap, mean reaction performance is poor compared to when no nap is taken, then improves across the remainder of the protocol.

On one occasion a participant made no correct matches or correct non-matches. In this instance mean reaction time could not be calculated. On another 19 occasions less than 5 correct matches or correct non-matches were made but mean reaction time was still calculated. When large numbers of incorrect matches are made and mean reaction time is calculated across only a small number of correct matches or correct non-matches, the resulting reaction time, when viewed in isolation, gives a potentially biased view of a participant's performance (i.e. that is their performance is better than what it actually is).

Differences From No Nap Condition

As was calculated for subjective sleepiness, the difference between performance when a nap was provided and performance when no nap was provided was determined at

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[2] Data at each test time were not always normally distributed

each test time to remove circadian effects. The differences for the total number of correct matches and non-matches, reaction time (correct matches plus correct non-matches), and number of omissions are plotted in Figure 8.

As can be seen in Figure 8, for all variables and all nap durations, performance immediately after waking from a nap (test battery 8) is poorer compared to when no nap is taken. Following a 20-minute nap, performance from test battery 9 onwards seems to improve and be better than, or similar to, performance when no nap is taken. Performance from test battery 9 following a 40-minute nap is similar to that when no nap is taken, except that reaction time performance remains slower than during the no nap condition. Reaction time performance following a 60-minute nap appears to be slower than when no nap is taken for approximately the first hour and then improve relative to the no nap condition. In contrast, the total number of correct matches and correct non-matches and number of omissions from test battery 9 following a 60-minute nap is similar to that when no nap is taken.
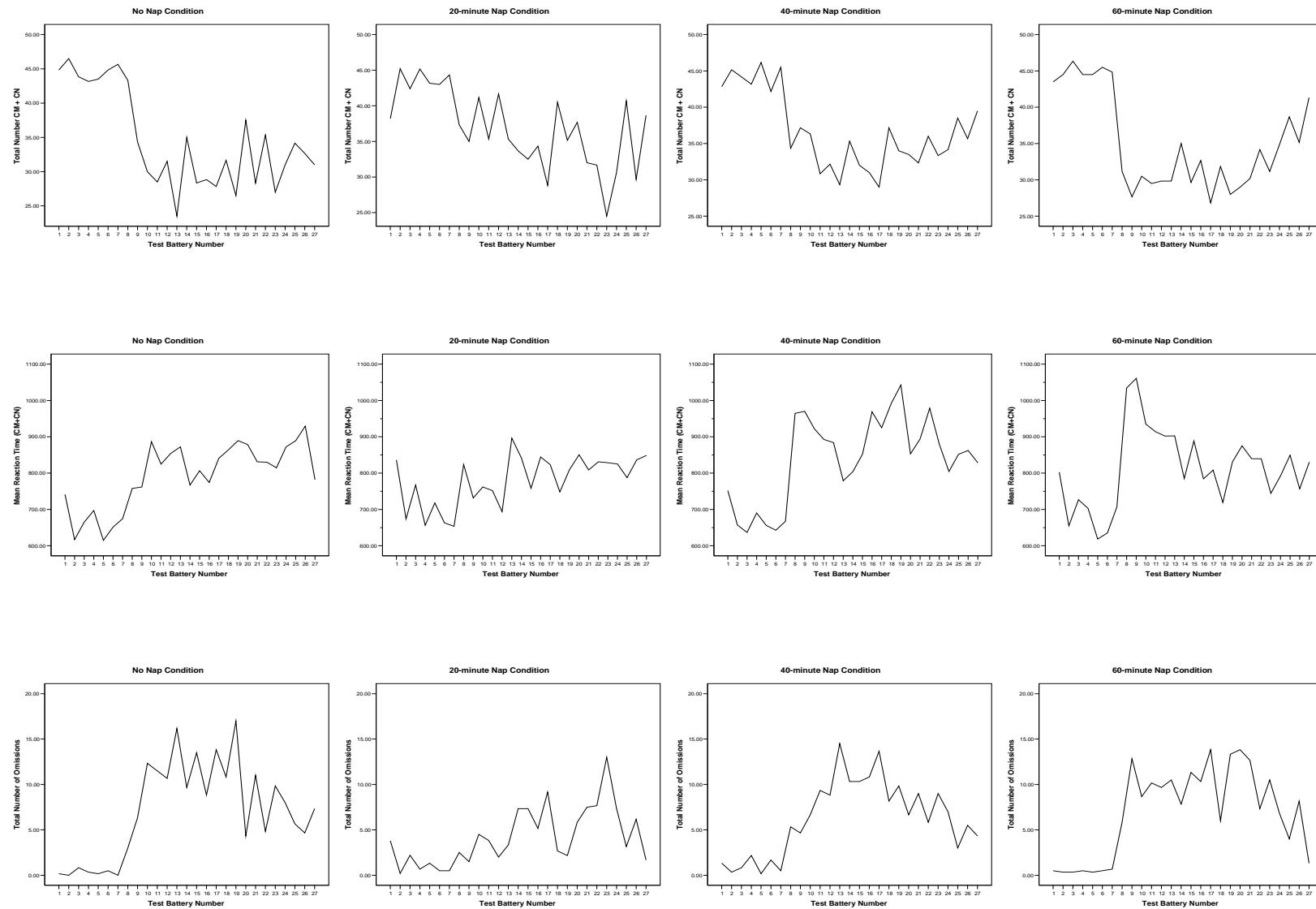
Figure 7. Means for number of CM plus CN, reaction time (CM plus CN) and number of omissions during each study condition.

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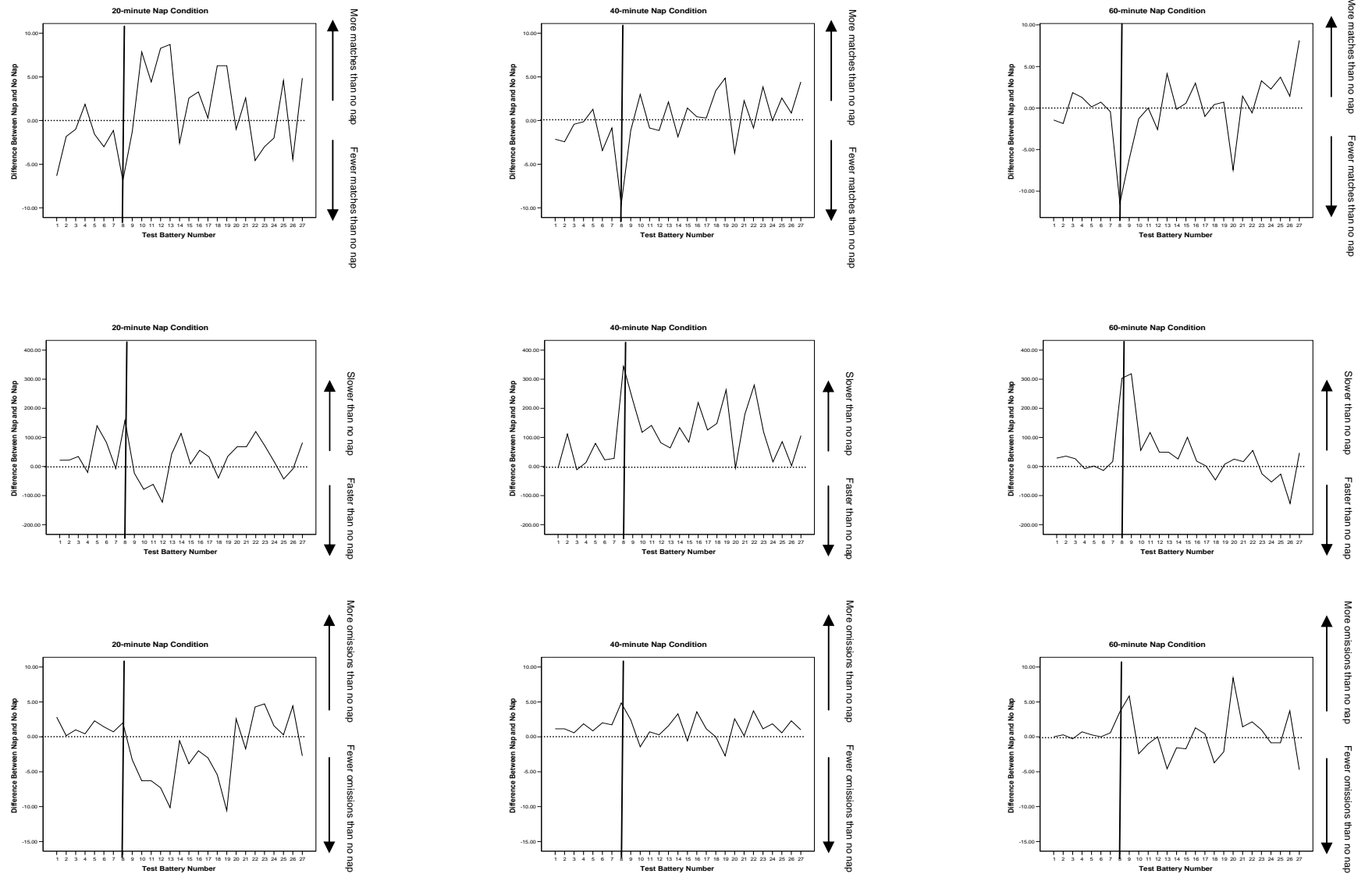**Figure 8**. **Differences from no nap for number of CM plus CN, reaction time (CM plus CN) and number of omissions during each study condition.**

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Discussion

The intention of this final report is to provide a summary of the processes used during the first 3 runs of the study and an overview of the data collected. Overall, the study has progressed as planned. The recruitment and screening of participants and the collection of data has been the focus of the research team to date.

Participant Recruitment and Retention

Six of the 22 individuals who enquired about the study were screened out by the questionnaire or did not meet the medical requirements (due to the medication they were taking), indicating the importance of the screening process in selecting individuals suitable for involvement in the study. Seven further individuals decided at some point during the screening process not to continue their involvement. Thus, 41% of individuals who initially indicated an interest in participating in the study were assigned to 1 of the first 3 runs. Of these, 78% completed all 4 weekends of data collection. Although the loss of 2 participants was for reasons unrelated to the study, the protocol is lengthy and complex and requires a great deal of commitment from participants. All attempts were made to ensure individuals were well informed about the study requirements and this level of attrition was not unexpected.

Nap Sleep

The structure of the nap sleep was as expected, with the short latencies to sleep, small amount of wake, and relatively large amount of SWS in the longer naps, suggesting participants had a high sleep drive and thus as the study design intended, maximising the likelihood of sleep inertia occurring. The increasing amount of SWS seen in the longer naps was as predicted and will allow us, in our final dataset, to investigate whether the duration of SWS or waking from SWS influences the occurrence of sleep inertia (after controlling for the duration of the nap). Also as anticipated, compared to sleep we have previously recorded in work settings (46) sleep efficiency during the nap opportunities was high. This will mean that our results will tend to be conservative, and possibly overestimate the effects of sleep inertia, compared to that occurring in operational settings (with the same duration of prior wake and napping at the same circadian phase).

Sleep Inertia Effects

Subjective sleepiness ratings collected to date suggest that both the 40-minute and 60-minute naps produce a sleep inertia effect, while performance data indicates that all naps may result in some degree of sleep inertia.

From the plots of data, sleep inertia appears to be more pronounced and last for longer following a 40-minute nap compared to the 60-minue nap. However, the small data set and possible confound of the order in which study conditions were completed may have contributed to this apparent relationship. This will be reexamined in the full data set.

It certainly appears, from the two measures considered in this report, that these facets of mood and functioning are sensitive enough to detect sleep inertia effects. We therefore predict that sleep inertia will also be measurable in the EEG data. It is also our intention to combine EEG indices with performance metrics, as it has been previously indicated that this can result in greater discriminability from baseline[3].

Effect of Nap Duration

The preliminary results also indicate that the duration of the nap has an effect on subjective perceptions of sleepiness subsequent to any sleep inertia effects dissipating. Both the 40-minute and 60-minute nap results in lower ratings of sleepiness towards the end of the protocol (across the early hours of the morning), with this effect appearing earlier and being stronger following a 60-minute nap. In contrast, decreased sleepiness following a 20-minute nap seems to be immediate but the effects are not maintained across the remainder of the protocol.

The positive benefits of the nap for working memory performance are, at this stage, much less apparent in the preliminary data. As with subjective sleepiness, the benefits of the 20-minute nap seem to be almost immediate but are not maintained across the remainder of the testing. There may be an improvement in performance following a 60-minute nap, but these effects are not obvious until late in the protocol. The 40-minute nap does not appear to have any observable benefit for performance. However, performance was highly variable between individuals and due to this variability and

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[3] Smith et al. (31) demonstrated that combining EEG spectral characteristics with the performance measures resulted in a monotonic increase in discriminability from baseline. In other words, using analysis of performance measures alone, 69 % data samples could be accurately discriminated from the baseline measure. In the combined analysis, 94% of the data samples could be accurately discriminated from baseline.

small data set, mixed model analyses controlling for factors such as the order of study conditions, have been postponed until the full data set is available.

Present and On-going Work

Data collection will be complete by the 2nd June 2007 and at that stage we hope to have complete data from 12 participants. Alongside the data collection process we have been working with a Biomedical Engineer to develop software to use for the analysis of the 90-second waking EEG recordings. This has been a lengthy process as we have carefully revisited the way in which we process this type of data. In previous research (47, 48) we employed a purpose built LabVIEW programme to manually remove all artefact from EEG data prior to undertaking spectral analysis. This process is very time consuming and results in the loss of a large amount of data. More recently, sophisticated digital signal processing techniques have become more widely available and accepted for artefact detection and removal. Our new software, based in Matlab, uses Independent Component Analysis (ICA) to remove eye movement and muscle artefact. Identifying and removing artefact by this method is faster and allows a much greater amount of EEG data to remain and be used for subsequent spectral analysis. The software development is complete and we will now begin the analysis of the 90-second recordings.

Additional future work includes scoring all remaining sleep recordings, from both the first night of the protocol and the nap opportunities, by two experienced sleep researchers. Final databases will then be prepared for all sleep, subjective sleepiness, performance, and EEG variables. It is our intention, as demonstrated in this final report, to use mixed model methods for statistical analyses. However, rather than linear models, as used in the present report, it is likely that non-linear methods will be employed. This will enable analysis of changes across the course of the protocol, in addition to the comparisons illustrated here between protocols for each test session.

In summary, data collection has proceeded as planned, scoring of nap sleep and waking EEG data are in progress, and we expect to complete the project on time by 31st October.

Appendix A: Participant Screening

Individuals willing to partake in the study were screened to determine whether they meet the selection criteria. The screening process consisted of 3 components: completing a screening questionnaire and meeting certain criteria on a number of standard scales; completing and passing a medical examination; and completing and passing a urine drug test prior to each weekend protocol.

Screening Questionnaire

The screening questionnaire, used in previous studies (49, 50) conducted at the Sleep/Wake Research Centre, was comprised of the following:

General Health Items

A questionnaire adapted from that used previously by Roach (51) includes questions about physical and mental health, smoking, alcohol consumption, overseas travel, level of stress, and napping. Additional health-related questions were also included from a New Zealand nationwide survey of Insomnia (52).

The Epworth Sleepiness Scale (ESS)(53) was included, which is a validated sleep questionnaire measuring daytime sleepiness by means of assessing the likelihood of falling asleep in 8 situations commonly encountered in daily life, namely: sitting and reading; watching television; sitting inactive in a public place; as a passenger in a car for an hour without a break; lying down in the afternoon when circumstances permit; sitting and talking to someone; sitting quietly after lunch without alcohol; and in a car while stopped for a few minutes in traffic. Possible scores range from 0 (no chance of dozing in any of the eight situations) to 24 (high chance of dozing in all eight situations). An ESS score greater than 10 indicates excessive daytime sleepiness.

Pittsburgh Sleep Quality Index (54)

This questionnaire assesses a person's sleep quality and disturbances during the past month. Nineteen individual items generate 7 component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. The sum of these component scores yields one global score. Those with a global score equal to or less than 5 are considered normal sleepers.

Depression-Anxiety-Stress Scales (DASS) (55)

This questionnaire measures a person's negative emotional states of depression, anxiety and stress over the past week. The half-length, 21-item, version (DASS21) was used in the present study, which contains 7 items for each scale.

Horne-Ostberg Morningness-Eveningness Questionnaire (56)

This questionnaire assesses a person's circadian type, by asking questions on preferential sleep timing and activities. The majority of questions are 4-choice items, which correspond to a definite morning type, moderate morning type, moderate evening type and definite evening type. Minor language modifications were made to suit the New Zealand population. The original MEQ scores (Definite Morning type: 70-86; Moderately Morning type: 59-69; Neither type: 42-58; Moderately Evening type: 31-41; Definite Evening type: 16-30) were used in the present study, as the participants' age range most closely resemble that of the student population used in the Horne-Ostberg Morningness-Eveningness Questionnaire validation study.

Medical Examination

Where answers to any questionnaire item suggested a health problem, the physician discussed this issue during the medical examination and decided whether the participant was suitable for inclusion in the study.

The medical examination was guided by a medical checklist, the purpose of which was to confirm the participant's self reported health status as indicated in the General Health Questionnaire and on the DASS, as well as ruling out any medical conditions that might be aggravated by participation in the study. The medical checklist was a modified version of one typically used by the local hospital. A 'Consent to Release Information' form was been included in the event that the physician detected a condition which required follow-up by the participant's General Practitioner or other health professional.

The physician carrying out the medical examination indicated on a Medical Results sheet whether or not the participant had any medical conditions, or was taking any medications that may affect his sleep and/or the study outcomes. Uncertain cases were discussed with the research team.

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Urine Drug Screen Test

Samples for urine drug testing were collected at the start of each of the weekend protocols. This test is required to rule out recent use of cannabis, amphetamines, benzodiazepines, opiates (including codeine, which is present in a number of cold and flu medications that are sold over the counter) and/or herbal party drugs (piperazines) that could affect the participant's level of alertness. All samples were analysed by the Institute of Environmental Science and Research Ltd (ESR), Porirua.

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